

# Ligand-Signaled Upregulation of *Enterococcus faecalis ace* Transcription, a Mechanism for Modulating Host-*E. faecalis* Interaction

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***Enterococcus faecalis*, the third most frequent cause of bacterial endocarditis, appears to be equipped with diverse surface-associated proteins showing structural-fold similarity to the immunoglobulin-fold family of staphylococcal adhesins. Among the putative *E. faecalis* surface proteins, the previously characterized adhesin Ace, which shows specific binding to collagen and laminin, was detectable in surface protein preparations only after growth at 46°C, mirroring the finding that adherence was observed in 46°C, but not 37°C, grown *E. faecalis* cultures. To elucidate the influence of different growth and host parameters on *ace* expression, we investigated *ace* expression using *E. faecalis* OG1RF grown in routine laboratory media (brain heart infusion) and found that *ace* mRNA levels were low in all growth phases. However, quantitative reverse transcription-PCR showed 18-fold-higher *ace* mRNA amounts in cells grown in the presence of collagen type IV compared to the controls. Similarly, a marked increase was observed when cells were either grown in the presence of collagen type I or serum but not in the presence of fibrinogen or bovine serum albumin. The production of Ace after growth in the presence of collagen type IV was demonstrated by immunofluorescence microscopy, mirroring the increased *ace* mRNA levels. Furthermore, increased Ace expression correlated with increased collagen and laminin adhesion. Collagen-induced Ace expression was also seen in three of three other *E. faecalis* strains of diverse origins tested, and thus it appears to be a common phenomenon. The observation of host matrix signal-induced adherence of *E. faecalis* may have important implications on our understanding of this opportunistic pathogen.**

*Enterococcus faecalis*, a common colonizer of the human intestinal tract, is also known to cause clinical disease, including bacteremia, urinary tract infections, and up to 15% of all cases of bacterial endocarditis (17). The ability of *E. faecalis* to colonize vascular tissue is thought to occur by adhesin-ligand interactions between its surface determinants and host proteins at the sites of endovascular injuries. To promote this colonization, *E. faecalis* possesses a number of predicted surface proteins with a characteristic immunoglobulin-like fold, which are called MSCRAMMs (for microbial surface components recognizing adhesive matrix molecules) (25). MSCRAMMs of staphylococci and streptococci have been reported to play a major role in adherence and colonization in animal models and, presumably, in humans (3), and it is likely that the same is true for enterococci.

Knowledge of the factors that influence the ability of *E. faecalis* to colonize host tissues is beginning to emerge. During the past decade, *E. faecalis* has been shown in various studies using different methodologies to adhere to one or more host extracellular matrix (ECM) proteins such as collagen types I and IV (CI and CIV), laminin (LN), fibronectin, lactoferrin, vitronectin, and thrombospondin (6). Using a standard in vitro adherence assay, Xiao et al. (29) reported that adherence of *E. faecalis* to collagen and LN was seen only after growth under a nonphysiological stress condition (i.e., at 46°C). Seemingly in contrast to this observation, Tomita et al. (27) recently dem-

onstrated collagen and LN adherence phenotypes of several *E. faecalis* clinical isolates by using a microscopic technique; however, this assay appears to be more sensitive than adherence studies that assess the percentage of bacteria bound.

By searching for homologues of known adhesins, Rich et al. (22) identified a gene in *E. faecalis* subsequently named *ace* (for adhesin of collagen from *E. faecalis*) and localized the specific CI binding property of Ace to the A domain based on biochemical evidence. Our further genetic analyses with an isogenic mutant demonstrated that Ace mediates the conditional (i.e., after growth at 46°C) adherence of *E. faecalis* to CIV and LN (19), in addition to dentin, a stabilized form of collagen (10). The study by Tomita et al. (27) that scored transposon insertion mutants of *E. faecalis* tissue-specific adhesive clinical strain also found that *ace* knockouts lacked CIV and LN adherence.

Our subsequent analyses of the *ace* gene from *E. faecalis* strains recognized that this gene is ubiquitous (2) and occurs in at least four different forms due to variation in the number of repeats of the B domain (20). Conditional in vitro production of Ace by different strains, detected by using polyclonal anti-Ace antibodies, was correlated with the conditional adherence of these *E. faecalis* strains to collagen and LN (20). Most recently, a role for Ace as a virulence factor was shown by using an arthritis model by expressing it in a surrogate host; in that study, Ace-expressing *Staphylococcus aureus* showed increased arthritogenic potential, to a level similar to that of *S. aureus* expressing Cna, a collagen-binding *S. aureus* homologue of Ace (30).

It is well known that bacteria can alter the expression of certain genes upon binding and replicating on a substrate, possibly via the mediation of various environment signals in-

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cluding collagen (1, 4, 9, 28). Previous studies have suggested that physiologically relevant cues, such as serum, may increase the adhesion of *E. faecalis* to heart cells (7, 8), to cultured renal tubular cells (11), and to CI (13). However, the specific signals that are sensed in serum remain largely unknown. An exploratory study by Shepard and Gilmore (24) compared mRNA levels of predicted *E. faecalis* virulence factors in cultures grown in serum, urine, or laboratory medium and identified environment- and growth-phase-specific variations in several virulence-related genes, including *ace*. However, an effect of these gene expression changes on phenotype(s) has yet to be elucidated. The present study was designed to examine *ace* transcription during in vitro growth conditions that mimic more physiological ones. Here, by measuring the levels of *ace* mRNA using quantitative reverse transcription-PCR (qRT-PCR), we demonstrate the upregulation of *ace* transcription when *E. faecalis* cultures were grown in the presence of CIV. Surface-localized Ace was detectable after growth in the presence of CIV, and it was correlated with increased adherence to CIV and LN.

#### MATERIALS AND METHODS

**Bacterial strains and growth conditions.** The *E. faecalis* strains used in the present study include OG1RF (18, 19) TX5256 (*ace* disruption mutant of OG1RF) (19); two endocarditis isolates, TX0052 and MC02152 (15, 20); and a urine isolate, MD9 (20). These strains were chosen based on clearly different pulsed-field gel electrophoresis patterns and different geographical origins. The *ace* mutant was constructed previously (19) by cloning an intragenic *ace* fragment in the suicide vector pTEX4577 containing *aph(3')-IIIa* (26). *E. faecalis* were routinely grown in brain heart infusion (BHI; Difco Laboratories, Detroit, Mich.) at 37°C, unless a different condition is specified.

**ECM proteins and collagenase digestion.** Bovine CI was purchased from Cohesion Technologies, Inc. (Palo Alto, Calif.), human-placenta-derived CIV was from Sigma Chemical Co. (St. Louis, Mo.), fibrinogen was from Enzyme Research Laboratories (South Bend, Ind.), and bovine serum albumin (BSA) was from MP Biomedicals, Inc. (Irvine, Calif.).

To eliminate collagen for certain reactions, CIV was suspended (0.5 mg/ml) in 50 mM Tris-HCl (pH 7.0) containing 40 mM CaCl<sub>2</sub> and then incubated at 10°C for 18 h with *Clostridium histolyticum* collagenase (Sigma Chemical Co.) at a substrate/enzyme ratio of 10:1 (14). The reaction mixture was dialyzed against 0.25% (wt/vol) acetic acid at 4°C for 4 days with five changes, freeze-dried, and resuspended in 0.25% (wt/vol) acetic acid.

**Gene expression analysis. (i) Extraction of total RNA.** Total RNA was isolated from *E. faecalis* cultures by using an RNeasy minikit (QIAGEN, Valencia, Calif.) according to the protocol of the supplier with some modifications. A lysozyme solution at 10 mg/ml was used instead of 3 mg/ml for the lysis step. Total RNA (20 to 40 µg) was treated three times with 20 U of RQ1 DNase (Promega Corp., Madison, Wis.) for 30 min at 37°C, and the DNase was removed by using the RNeasy minikit. The RNA concentration was determined by using a spectrophotometer. Part of each sample was electrophoresed through an agarose-formaldehyde gel in morpholinepropanesulfonic acid buffer as previously described (23).

**(ii) RT-PCR.** Total RNA (between 5 ng to 250 ng) was reverse transcribed with *ace* specific primers (AceMF, 5'-ACGATTGAAGGAGTGACTAACACA-3'; AceMR; 5'-AAGTGTAACGGACGATAAAGGAAG-3') using the SuperScript One-Step RT-PCR with a Platinum Taq kit (Invitrogen Corp., Carlsbad, Calif.) according to the manufacturer's instructions. As an internal control, a 528-bp fragment of *gdh* (encoding GAPDH [glyceraldehyde-3-phosphate dehydrogenase]) was amplified by using the *gdhF* (5'-AGTGGCGCACTAAAAGATATG G-3') and *gdhR* (5'-AGTTGTATTGAACCCCTTGACCG-3') primers. Reactions without reverse transcriptase were performed as controls to detect DNA contamination in the total RNA preparations.

**(iii) Real-time qRT-PCR.** Amplification, detection, and real-time analysis were performed by using the ABI Prism 7500 sequence detection system (Applied Biosystems, Foster City, Calif.). Primers designed to produce amplicons of equivalent length were selected by using Primer Express software (Applied Biosystems). The primer pairs used in qRT-PCR included AceQF1 (5'-GGAGAGTC AAATCAAGTACGTTGGTT-3')-AceQR1 (5'-TGTTGACCACTTCCTTGTC

GAT-3') and 23S-rRNA (5'-GTGATGGCGTGCCTTTTGTGA-3')-23S-rRNA (5'-CGCCCTATTACAGACTCGCTTT-3'). For each sample, cDNA synthesis and PCR amplification were performed in a two-step process. For cDNA synthesis, 4 µg of total RNA was added to 20-µl reaction solution containing 40 U of RNase OUT, and RT reactions were performed with random primers and SuperScript II reverse transcriptase (Invitrogen). The reaction was stopped by heating at 70°C for 15 min. After the RNA complementary to the cDNA was removed with *E. coli* RNase H (Invitrogen), the cDNA was purified by using QiaQuick PCR purification kit (QIAGEN). The resulting cDNA diluted up to 500 times was used for subsequent PCR amplification with the appropriate forward and reverse gene specific primers and the SYBR Green PCR Master Mix kit (Applied Biosystems). The following PCR conditions were used: 10 min at 95°C for the initial denaturation, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. All PCR fragments yielded a single band on an agarose gel. Relative quantification of gene expression was performed by using 23S rRNA mRNA as the internal standard. The  $\Delta\Delta C_T$  method (12) was used to calculate the relative amount of specific RNA present in a sample, from which the fold induction of transcription of the gene was estimated by comparing to the values of OG1RF grown in BHI. The data were expressed as the mean  $\pm$  the standard deviation. The statistical significance was determined by using the Student unpaired *t* test. Amplifications were performed on four independent RNA samples from each milieu.

**Immunofluorescence microscopy.** *E. faecalis* cells either cultured in the presence or absence of ECM proteins in BHI or grown in 40% horse serum in BHI were washed thrice with Dulbecco phosphate-buffered saline (D-PBS) without CaCl<sub>2</sub> and MgCl<sub>2</sub> and then resuspended in D-PBS to a final optical density at 600 nm (OD<sub>600</sub>) of 0.5. Next, 400 µl of cell suspensions were applied to Lab-Tek-II chamber slides (Nalge Nunc International Corp., Naperville, Ill.) that were coated with poly-D-lysine (Sigma) and incubated for 30 min at room temperature with shaking at 100 rpm. After four washes with D-PBS, chamber slides were blocked with 1 ml of D-PBS containing 2% BSA at room temperature for 45 min, washed once with D-PBS, and then incubated with 400 µl of a 1:3,000 dilution for anti-Ace polyclonal serum (19) for 1 h. After four washes with D-PBS, attached bacteria in chamber slides were incubated with 400 µl of rhodamine red-labeled goat anti-rabbit immunoglobulin G (1:1,000 dilution) (Molecular Probes, Eugene, Oreg.) at room temperature for 1 h in the dark. After four washes, excess D-PBS was removed and a coverslip was mounted. The slides were examined by epifluorescence microscopy using an Olympus BX51 microscope with a  $\times$ 100 oil immersion objective lens (Olympus, Tokyo, Japan). Digital images were acquired by using an Olympus DP-70 digital camera. Preimmune serum was used as a negative control.

**Adherence assay.** Adherence of *E. faecalis* to CIV- and LN-precoated six-well plates (Becton Dickinson Biosciences, Bedford, Mass.) was determined by using an Olympus BX51 microscope. Each well of ECM-precoated plates were blocked with 5 ml of 0.2% BSA in PBS, incubated at 4°C for 2 h, and then washed with PBS three times. Cell pellets from *E. faecalis* cells either cultured in the presence or in the absence of ECM proteins were washed two times in PBS and resuspended in 0.1% Tween 80–0.1% BSA in PBS. The cell density was adjusted to an OD<sub>600</sub> of 0.2, and 1 ml of bacteria was added into each well, followed by incubation at room temperature for 2 h with gentle shaking at 70 rpm. Wells were washed three times with 0.1% Tween 80–0.1% BSA in PBS. The numbers of bacterial cells that adhered to the surface of the ECM were counted from 10 randomly chosen fields of vision. Each experiment was performed four times. Statistical analysis was determined by using the Mann-Whitney test, and the differences were considered significant when the *P* value was  $<0.05$ .

#### RESULTS AND DISCUSSION

The pathogenicity of *E. faecalis* may be associated with the expression of specific bacterial MSCRAMMs in response to environmental cues. Ace is one such protein with the typical characteristics of an MSCRAMM, exhibiting CI, CIV, and LN binding (19). Although Ace was not detectable on Western blots after growth in routine laboratory growth conditions, Ace-specific antibodies were found in sera obtained from patients with *E. faecalis* endocarditis, indicating that Ace is expressed in vivo during infection in humans and not just at 46°C in vitro (20). However, it has not previously been shown whether the in vitro conditional (46°C) Ace expression is mediated at the transcriptional or posttranscriptional level. In

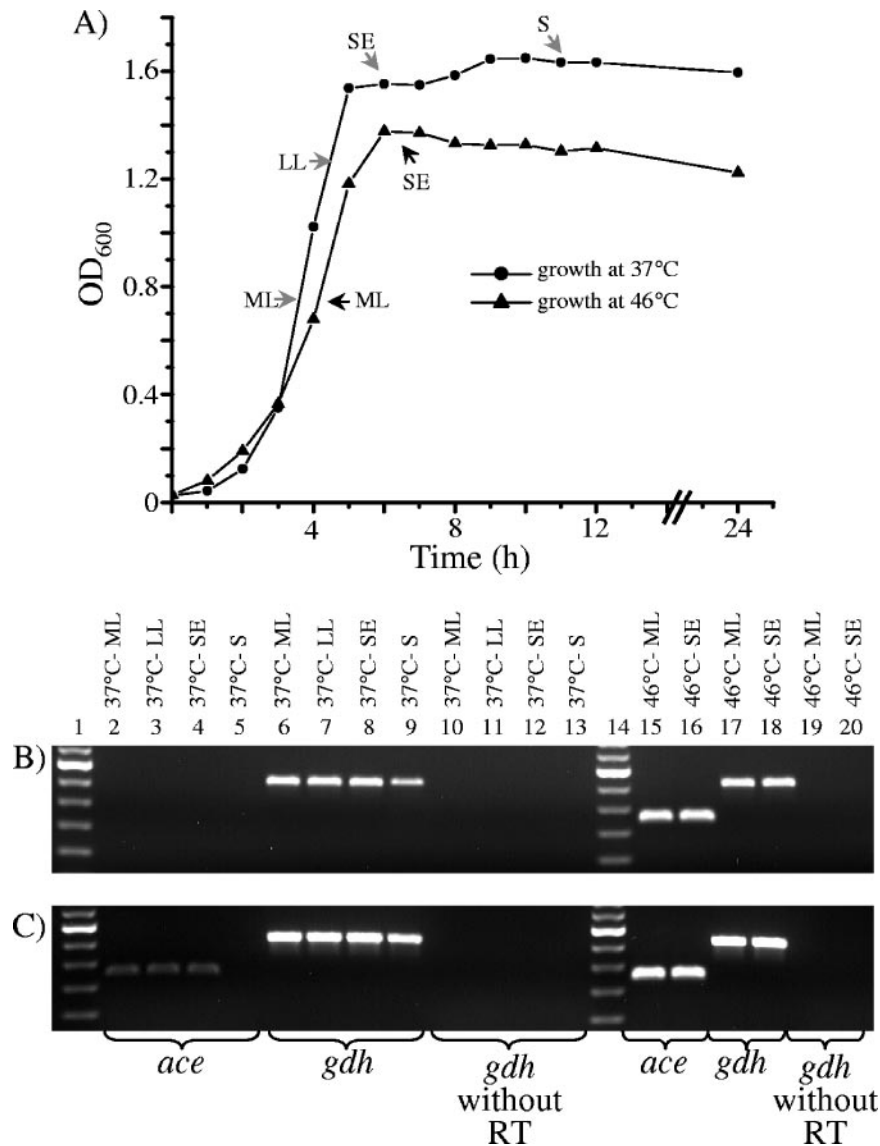


FIG. 1. RT-PCR analysis of *ace* transcripts in *E. faecalis* OG1RF. (A) Growth patterns of OG1RF at 37 or 46°C. Time points at which total RNA was isolated are marked with gray (37°C) and black (46°C) arrowheads. (B and C) RT-PCR-amplified bands for *ace* or control gene *gdh*. The total amounts of RNA per reaction used were 5 ng for each of the samples in panel B and 250 ng for each of the samples in panel C. A 100-bp DNA ladder (Invitrogen) was used as the size marker (lanes 1 and 14). For the samples in lanes 10 to 13, as well as lanes 19 and 20, the reverse transcriptase was omitted (negative control for RT). Growth temperatures, as well as different growth phases from which RNA was extracted, are given above each lane of panel B, and the genes analyzed are shown below panel C. ML, mid-log phase; LL, late-log phase, SE, stationary-phase entry; and S, stationary phase.

order to determine whether, in fact, the *ace* gene is transcribed during *in vitro* growth of *E. faecalis* strain OG1RF at 37°C in BHI, semiquantitative RT-PCR analysis was performed.

#### Minimal transcription of *ace* after growth *in vitro* in BHI.

Total RNA of OG1RF grown at 37°C in BHI was isolated during mid-exponential phase, late-exponential phase, entry into stationary phase, and 5 h after the cultures entered stationary phase (Fig. 1A), and the levels of *ace* and *gdh* (house-keeping control) mRNA were analyzed. The *ace* mRNA was barely detected at the mid-exponential and late exponential phases, as well as at entry into stationary phase, and was not detectable in cells at stationary phase (Fig. 1B and C, lanes 2 to 5). As anticipated, the control *gdh* gene was constitutively

expressed in all phases, although, at stationary phase, *gdh* mRNA levels were slightly reduced (Fig. 1B and C, lanes 6 to 9). This result suggests that *ace* transcription is very low during standard *in vitro* growth conditions. We next analyzed the RNA of OG1RF grown at 46°C isolated at mid-exponential phase and at entry into stationary phase (Fig. 1A). Increased *ace* mRNA was detected at both growth phases (Fig. 1B and C, lanes 15 and 16) and the *ace* RT-PCR band intensities were almost comparable to control *gdh* RT-PCR band intensities at all of the concentrations of total RNA tested (Fig. 1B and C, lanes 17 and 18). These results are in agreement with our previous findings of detectable levels of Ace from mutanolysin surface extracts (19) of OG1RF grown at 46°C.

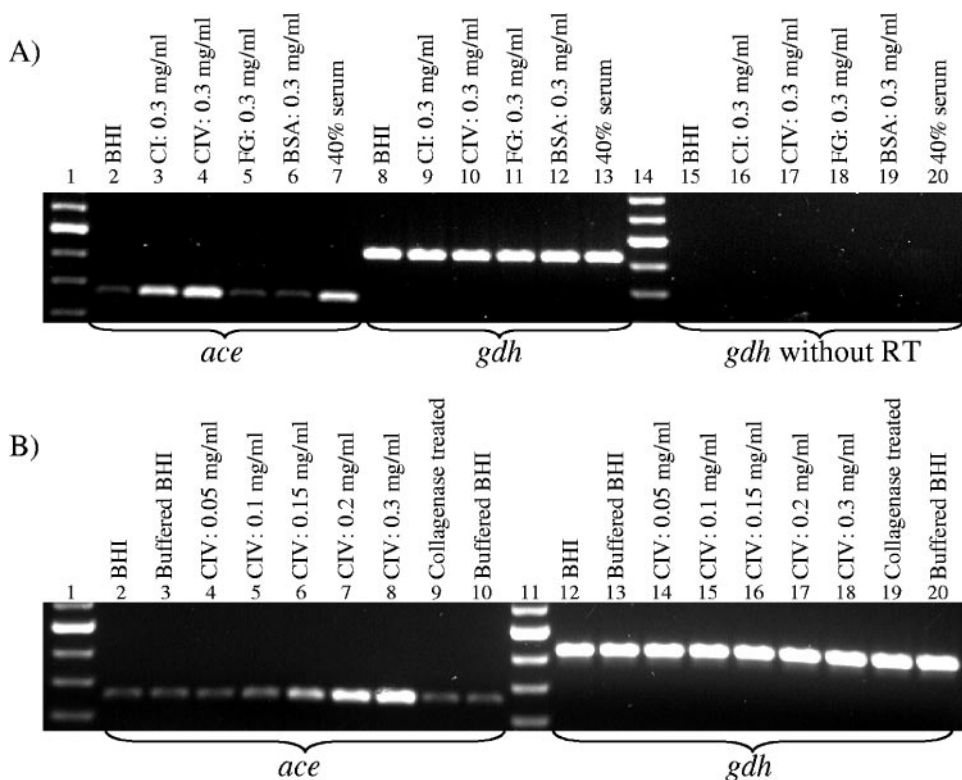


FIG. 2. *E. faecalis* OG1RF *ace* mRNA expression after growth in the presence of ECM proteins. The results are presented as amplified products electrophoresed on ethidium bromide-stained agarose gels. RT-PCR amplification of the *gdh* (housekeeping gene) was performed to ensure that similar amounts of input RNA and similar RT efficiencies were being compared. (A) Effect of different ECM proteins and serum on *ace* transcription. Mid-exponential-growth-phase cultures in BHI medium were split into aliquots and incubated with 0.3 mg of CI, CIV, fibrinogen (FG), or BSA/ml. Samples were removed after 1 h, and total RNA was prepared as described in Materials and Methods. For cultures grown in 40% horse serum in BHI, total RNA was extracted from the late exponential growth phase. (B) Dose-dependent induction of *ace* expression by OG1RF. OG1RF cells were grown in the presence of 0.05 mg/ml to 0.3 mg of CIV/ml for 1 h. The samples in lanes 3 and 10 of panel B are RT-PCR amplicons of RNA isolated from cultures grown in BHI buffered with CH<sub>3</sub>COOH and HCl, respectively. RT-PCRs performed with two independent sample preparations showed similar results.

***ace* transcription in *E. faecalis* OG1RF is upregulated after growth in the presence of collagen.** Since there is a precedent from other gram-positive organisms of upregulation of specific genes upon exposure to the ligand of their products (9), we explored this possibility by investigating *ace* mRNA levels from bacteria grown in the presence of different ECM proteins. Initially, BHI-broth-grown, mid-exponential-growth-phase (OD<sub>600</sub> = 0.7 to 0.8) cultures of OG1RF were supplemented with 0.03 and 0.3 mg of CIV/ml and cultured further for various post-exposure times (15 min, 30 min, 45 min, 1 h, 2 h, and 3 h). RT-PCR analysis of these total RNA preparations showed that the addition of CIV for 30 min at a concentration of 0.3 mg/ml resulted in increased *ace* mRNA amounts from the minimal levels seen without CIV and that the maximum increase was seen at 1 h (data not shown). To test the effects of different ECM proteins on *ace* transcription, mid-exponential-growth-phase cultures were divided into aliquots and incubated for 1 h (about two generation times) in the presence or absence of 0.3 mg/ml of CI, CIV, fibrinogen, or BSA. As depicted in Fig. 2A, the addition of CI caused a clear increase in the *ace* transcript levels (lane 3), and the addition of CIV (to which Ace has maximum affinity) resulted in much higher levels of *ace* mRNA (lane 4), whereas the addition of fibrinogen or BSA had no

effect on *ace* mRNA levels (lanes 5 and 6). Furthermore, we have also demonstrated that CIV-mediated *ace* induction of OG1RF is observed only in actively growing culture, but not in the cells suspended in PBS (data not shown), as they are for the adherence assay. Importantly, enhancement of *ace* mRNA levels observed in the presence of CIV occurred in a dose-dependent manner that paralleled the amount of CIV added (Fig. 2B, lanes 4 to 8). These results indicate that differential *ace* gene expression depends on the milieu and that the presence of collagen may act as a signal for *ace* regulation.

**Effect of pH on *ace* expression.** During the course of our experiments, we noticed that the final pH of the culture medium was reduced from pH 7.3 to pH 6.4 or 6.7 upon the addition of CIV or CI, respectively. To test whether low extracellular pH could itself affect expression of *ace* in the absence of collagen, we measured the expression of *ace* in BHI medium buffered to the same pH values using acetic acid or HCl, respectively. At this pH, there was no significant difference in the level of *ace* mRNA (Fig. 2B, lanes 3 and 10), although there is a slight decrease in the final OD of the cultures after growth in this buffered medium. This result indicates that the increase in *ace* transcripts seen in collagen-supplemented medium is not due to the change in pH.

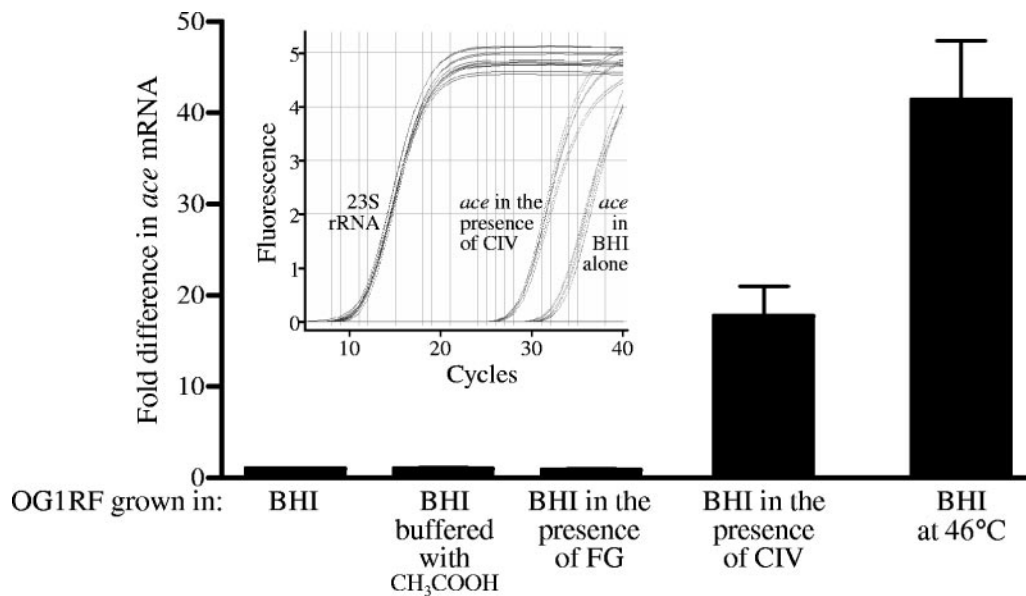


FIG. 3. Quantitative *ace* mRNA levels in *E. faecalis* OG1RF exposed to different milieu. Relative levels of *ace* transcripts from OG1RF grown in the presence or absence of CIV or FG or grown at 46°C were quantified with reference to transcripts of OG1RF grown in BHI at 37°C. The 23S rRNA transcript was used as an endogenous control. Primer pairs were checked for primer-dimer formation by dissociation curve and also by using the primers without the addition of RNA template. The results are presented as means  $\pm$  the standard deviations. In the insert, the real-time amplification plots of RNA samples obtained from cultures grown in the presence or absence of CIV were shown.

**Intact collagen peptides but not collagenase-derived digests induce *ace* expression.** To investigate whether induction of *ace* transcripts was associated with the structure and/or sequence of collagen peptides or with its primary repeat tripeptides (Gly-X-Y), RT-PCR was carried out by using cultures incubated with collagenase-digested CIV. No change in transcript levels of *ace* was observed, and the levels remained similar to those for BHI-grown cells (Fig. 2B, lane 9), suggesting that induction of the *ace* transcripts was associated with intact collagen peptides.

**A serum-rich environment affects the expression of *ace* gene.** In an effort to identify other physiological conditions that up-regulate *ace* expression, we tested *ace* transcript amounts after growth in BHI supplemented with serum. As shown in Fig. 2A, lane 7, growth in 40% horse serum increased the levels of *ace* mRNA. This observation is in agreement with a previous study (24) that showed a 3.3-fold increase in *ace* mRNA abundance upon growth in serum compared to growth in 2xYT (yeast extract, tryptone, sodium chloride) medium. Of note, growth in serum resulted in the formation of aggregates both in OG1RF and in TX5256 (*ace* mutant), indicating that this phenomenon is not dependent upon Ace.

**Quantification of *ace* transcript induction.** To measure the fold differences in *ace* transcription, real-time qRT-PCR was used. The transcription level of 23S rRNA was not significantly affected by growth under any of the conditions tested and, hence, was used for normalization. Comparisons were made between values obtained from the total RNA of *E. faecalis* cultured in the presence CIV, fibrinogen, or growth at 46°C relative to those from BHI cultures grown at 37°C. Thus, *ace* mRNA after growth in BHI at 37°C was normalized to a value of 1 as the baseline for comparison. The results showed that there is an 18-fold increase in the *ace* mRNA levels in cultures

grown in BHI containing CIV compared to cells grown in BHI alone (Fig. 3). As anticipated from semiquantitative RT-PCR, the levels of *ace* mRNA was not altered either in the presence of fibrinogen or by pH. Furthermore, *ace* mRNA levels of 46°C grown cells was observed to be 2.3-fold more than the levels of cells grown in the presence of CIV (41-fold versus BHI).

**Modulation of Ace expression by collagen type IV in diverse *E. faecalis* strains.** To determine whether increased *ace* mRNA is correlated with Ace protein expression, *E. faecalis* OG1RF cells were grown in conditions similar to those described above, except that cells were grown for four generations after supplementation with ECM proteins. Cells were then stained with polyclonal anti-Ace antibodies, followed by rhodamine red-labeled secondary antibody; these anti-Ace antibodies have been previously shown to specifically react with Ace on Western blots (20). Analysis of surfaces of cells by fluorescence microscopy showed that anti-Ace serum stained 100% of cells grown in the presence of CI and CIV (Fig. 4D and F) but did not stain the cells grown in the presence of fibrinogen (Fig. 4H). Surface staining was also observed in cells grown in 40% horse serum in BHI (Fig. 4J) and in cells grown at 46°C (Fig. 4L). As expected, Ace antibodies failed to stain the surfaces of the similarly grown *ace* disruption mutant of OG1RF (TX5256) (data not shown).

Immunofluorescence-based localization of Ace was further carried out with three diverse clinical *E. faecalis* strains. For the endocarditis strain MC02152, staining was seen on some cells grown in BHI at 37°C (Fig. 4N), a finding consistent with this strain having previously been shown to produce low amounts of Ace (seen as a weak band in Western blot analyses) (20) after growth in BHI at 37°C. However, upon culturing of this strain in the presence of CIV, 100% of the cells were stained with anti-Ace (Fig. 4P). Like OG1RF, TX0052 (endo-

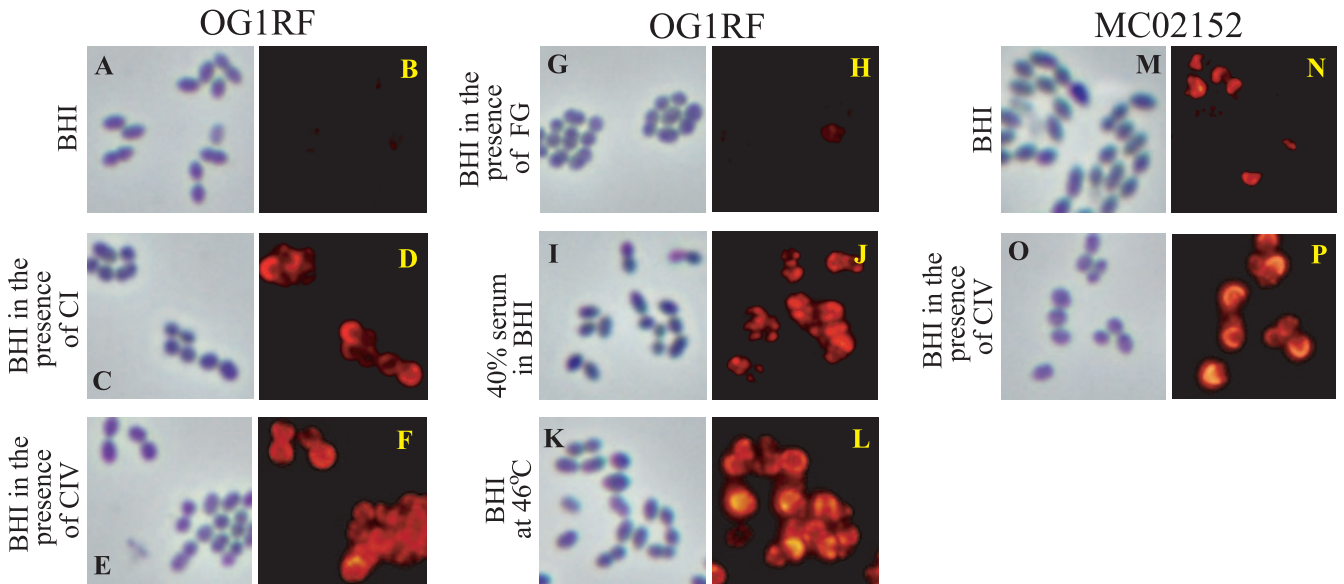


FIG. 4. Immunofluorescence images of surface-exposed Ace on *E. faecalis* strains. Panels A, C, E, G, I, and K (phase contrast) and B, D, F, H, J, and L (fluorescent visualization) are *E. faecalis* OG1RF cells stained with anti-Ace rabbit sera. Panels M and O (phase contrast) and N and P (fluorescent visualization) are *E. faecalis* strain MC02152 cells stained with anti-Ace rabbit sera. Rhodamine red-labeled goat anti-rabbit antibodies were used as the secondary antibody. Culture conditions are marked on the side of the respective panel pair. Preimmune rabbit sera with OG1RF and anti-Ace sera with *ace* mutant (TX5256) served as controls (data not shown).

carditis isolate) and MD9 (urine isolate) cell surfaces showed conditional staining (i.e., after culturing these strains in BHI containing CIV but not after culture in BHI alone). The finding of CIV-induced Ace expression in four of four diverse

strains tested suggests this may be a common phenomenon among *E. faecalis*.

**Collagen- and laminin-binding ability of *E. faecalis* as a function of upregulated *ace* expression.** *E. faecalis* OG1RF and

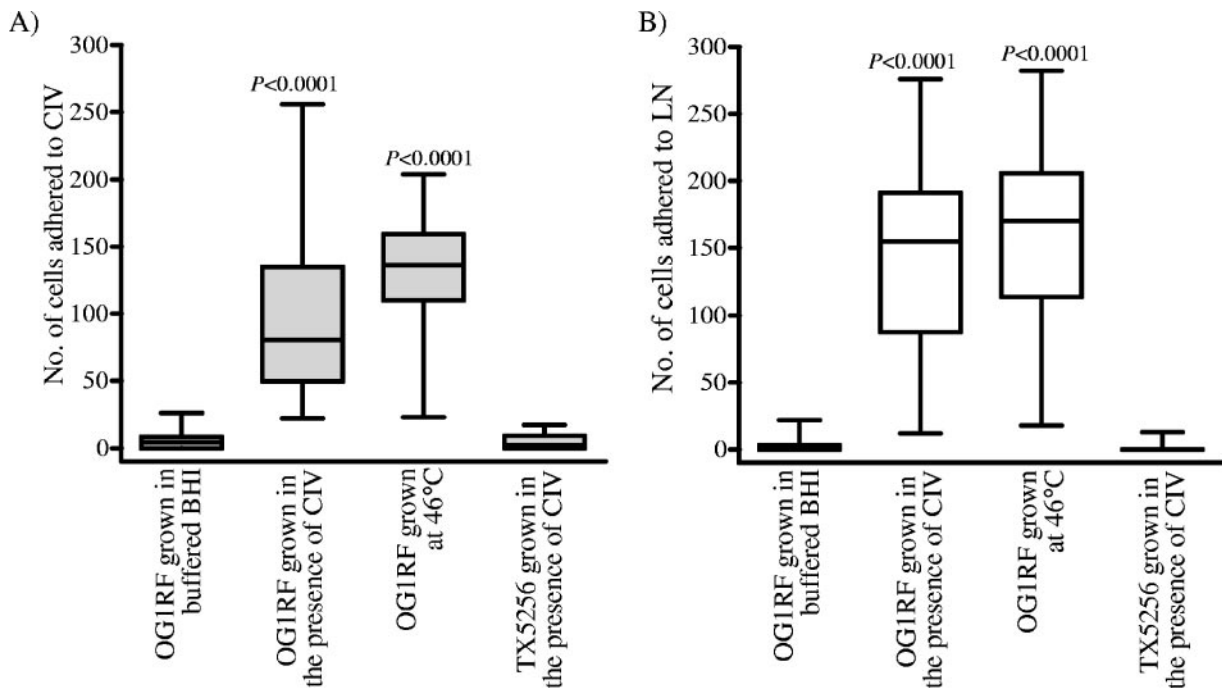


FIG. 5. Adherence assay with wild-type *E. faecalis* strain OG1RF and its isogenic *ace* disruption mutant (TX5256). The data shown in panels A and B represent the adherence of OG1RF cells to CIV and LN, respectively. Median and interquartile range values are shown. Adherent bacterial cells quantified by using 60 fields of phase-contrast microscope represent four independent experiments. Sample medians generated from the adherent bacteria quantifications were compared by using the Mann-Whitney test. Tests were performed by using GraphPad Prism v4 for Windows.

its isogenic *ace* disruption mutant, TX5256, were tested for their ability to adhere to immobilized CIV and LN by using microscopy. The cells of OG1RF grown in BHI in the presence of CIV, but not in those grown in BHI alone, adhered to CIV (Fig. 5A) and LN (Fig. 5B), whereas the *ace* mutant (TX5256) was completely defective in adherence to CIV and LN regardless of its growth conditions. This corroborates our earlier observation that Ace mediates the adherence of *E. faecalis* OG1RF grown at 46°C to immobilized CIV and LN. Thus, these results demonstrate that *E. faecalis* adhesion is enhanced directly in response to a host matrix-derived peptide signal. This mechanism of modulation of *E. faecalis*-collagen interaction is different from the mechanisms reported in *S. aureus* (5) and *E. faecium* (21) to alter their collagen adherence.

After growth in 40% horse serum, wild-type OG1RF adhered to immobilized CI, CIV, and LN in our standard radioactive adherence assay (19). Serum-induced CI adherence results are consistent with an earlier study with *E. faecalis* strain JH2-2 (13). As anticipated, the *ace* mutant of OG1RF (TX5256) showed markedly reduced adherence to CIV (from 18.1 to 4.2%) and LN (9.2 to 1.2%) compared to the wild type. However, adherence to CI was only partially reduced (from 47.3 to 31.4%), raising the possibility of the presence of an additional collagen-binding protein with high affinity to CI in *E. faecalis* OG1RF. Our recent observation with a *salB* mutant of OG1RF that exhibited only CI and fibronectin binding after growth at 37°C (16), corroborates there being a difference between CI and CIV adherence. Furthermore, immunofluorescence microscopy studies with Ace antibodies confirmed that the *salB* mutant of OG1RF lacks Ace on its surface (data not shown).

Although the ability to adhere to the most abundant host protein, collagen, would intuitively appear to be beneficial to *E. faecalis* during colonization, it may be of a selective disadvantage during growth in the environment, during transmission or dissemination of infection, and also during chronic infection to avoid the immune response. Therefore, we speculate that *E. faecalis* may have developed this strategy of programmed response to express proteins such as Ace.

In summary, upregulation of *ace* gene transcription in the presence of collagen type IV in *E. faecalis* OG1RF was demonstrated. Although we have yet to delineate the precise mechanism, we have confirmed that expression of Ace, and thereby collagen and laminin adherence, occurs under more physiological conditions than growth at 46°C. Ace induction by other environmental conditions, such as growth in serum and growth at high temperature, suggests the possibility that *ace* gene expression may be regulated by different mechanisms. Conservation of this induction mechanism in four of four different strains tested suggests that this may be a common programmed response in *E. faecalis*. Furthermore, understanding of this host matrix protein-associated triggering of a microbial pathogenic response may have important future clinical implications for this emerging multiresistant pathogen.

#### ACKNOWLEDGMENTS

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